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Barbet , et al. **June 26, 2001**

Nucleic acid vaccines for ehrlichia chaffeensis and methods of use

Abstract

Described are nucleric acid vaccines containing genes to protect animals or humans against Ehrlichia chaffeensis. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

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Goverment Interests

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Parent Case Text

CROSS-REFERENCE TO A RELATED APPLICATION

This is a continuation-in-part of U.S. patent application Ser. No. 08/733,230, filed Oct. 17, 1996 now U.S. Pat. No. 6,025,338.

Claims

What is claimed is:

1. A method of inducing an immune response to a rickettsial polypeptide comprising the amino

acid sequence of SEQ ID NO:23 or SEQ ID NO: 24 in an animal comprising the administration of a composition comprising a pharmaceutically acceptable carrier and nucleic acid vaccine vector containing an operably linked isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24, wherein said composition is administered in an amount effective to elicit an immune response.

2. The method, according to claim 1, wherein said polypeptide has the sequence shown in SEQ ID NO:23.

3. The method, according to claim 1, wherein said polypeptide has the sequence shown in SEQ ID NO:24.

4. The method, according to claim 1, wherein said nucleic acid further comprises a nucleic acid vector.

5. An isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24.

6. A composition comprising an isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24 and a pharmaceutically acceptable carrier.

7. A vector comprising an isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24.

8. A composition comprising a vector containing an isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24 and a pharmaceutically acceptable carrier.

9. The composition of claim 8, wherein said vector is a vaccine vector.

Description

TECHNICAL FIELD

This invention relates to nucleic acid vaccines for rickettsial diseases of animals, including humans.

BACKGROUND OF THE INVENTION

The rickettsias are a group of small bacteria commonly transmitted by arthropod vectors to man and animals, in which they may cause serious disease. The pathogens causing human rickettsial diseases include the agent of epidemic typhus, Rickettsia prowazekii, which has resulted in the deaths of millions of people during wartime and natural disasters. The causative agents of spotted fever, e.g., Rickettsia rickettsii and Rickettsia conorii, are also included within this group. Recently, new types of human rickettsial disease caused by members of the tribe

Ehrlichiae have been described. Ehrlichiae infect leukocytes and endothelial cells of many different mammalian species, some of them causing serious human and veterinary diseases. Over 400 cases of human ehrlichiosis, including some fatalities, caused by Ehrlichia chaffeensis have now been reported. Clinical signs of human ehrlichiosis are similar to those of Rocky Mountain spotted fever, including fever, nausea, vomiting, headache, and rash.

Heartwater is another infectious disease caused by a rickettsial pathogen, namely Cowdria ruminantium, and is transmitted by ticks of the genus Amblyomma. The disease occurs throughout most of Africa and has an estimated endemic area of about 5 million square miles. In endemic areas, heartwater is a latent infection in indigenous breeds of cattle that have been subjected to centuries of natural selection. The problems occur where the disease contacts susceptible or naive cattle and other ruminants. Heartwater has been confirmed to be on the island of Guadeloupe in the Caribbean and is spreading through the Caribbean Islands. The tick vectors responsible for spreading this disease are already present on the American mainland and threaten the livestock industry in North and South America

In acute cases of heartwater, animals exhibit a sudden rise in temperature, signs of anorexia, cessation of rumination, and nervous symptoms including staggering, muscle twitching, and convulsions. Death usually occurs during these convulsions. Peracute cases of the disease occur where the animal collapses and dies in convulsions having shown no preliminary symptoms. Mortality is high in susceptible animals. Angora sheep infected with the disease have a 90% mortality rate while susceptible cattle strains have up to a 60% mortality rate.

If detected early, tetracycline or chloramphenicol treatment are effective against rickettsial infections, but symptoms are similar to numerous other infections and there are no satisfactory diagnostic tests (Helmick, C., K. Bernard, L. D'Angelo [1984] J. Infect. Dis. 150:480).

Animals which have recovered from heartwater are resistant to further homologous, and in some cases heterologous, strain challenge. It has similarly been found that persons recovering from a rickettsial infection may develop a solid and lasting immunity. Individuals recovered from natural infections are often immune to multiple isolates and even species. For example, guinea pigs immunized with a recombinant R. conorii protein were partially protected even against R. rickettsii (Vishwanath, S., G. McDonald, N. Watkins [1990] Infect. Immun. 58:646). It is known that there is structural variation in rickettsial antigens between different geographical isolates. Thus, a functional recombinant vaccine against multiple isolates would need to contain multiple epitopes, e.g. protective T and B cell epitopes, shared between isolates. It is believed that serum antibodies do not play a significant role in the mechanism of immunity against rickettsia (Uilenberg, G. [1983] Advances in Vet. Sci. and Comp. Med 27:427-480; Du Plessis, Plessis, J. L. [1970] Onderstepoort J. Vet. Res. 37(3):147-150).

Vaccines based on inactivated or attenuated rickettsiae have been developed against certain rickettsial diseases, for example against R. prowazekii and R. rickettsii. However, these vaccines have major problems or disadvantages, including undesirable toxic reactions, difficulty in standardization, and expense (Woodward, T. [1981] "Rickettsial diseases: certain unsettled problems in their historical perspective," In Rickettsia and Rickettsial Diseases, W. Burgdorfer and R. Anacker, eds., Academic Press, New York, pp. 17-40).

A vaccine currently used in the control of heartwater is composed of live infected sheep blood.

This vaccine also has several disadvantages. First, expertise is required for the intravenous inoculation techniques required to administer this vaccine. Second, vaccinated animals may experience shock and so require daily monitoring for a period after vaccination. There is a possibility of death due to shock throughout this monitoring period, and the drugs needed to treat any shock induced by vaccination are costly. Third, blood-borne parasites may be present in the blood vaccine and be transmitted to the vaccinates. Finally, the blood vaccine requires a cold chain to preserve the vaccine.

Clearly, a safer, more effective vaccine that is easily administered would be particularly advantageous. For these reasons, and with the advent of new methods in biotechnology, investigators have concentrated recently on the development of new types of vaccines, including recombinant vaccines. However, recombinant vaccine antigens must be carefully selected and presented to the immune system such that shared epitopes are recognized. These factors have contributed to the search for effective vaccines.

A protective vaccine against rickettsiae that elicits a complete immune response can be advantageous. A few antigens which potentially can be useful as vaccines have now been identified and sequenced for various pathogenic rickettsia. The genes encoding the antigens and that can be employed to recombinantly produce those antigen have also been identified and sequenced. Certain protective antigens identified for R. rickettsii, R. conorii, and R. prowazekii (e.g., rOmpA and rOmpB) are large (>100kDa), dependent on retention of native conformation for protective efficacy, but are often degraded when produced in recombinant systems. This presents technical and quality-control problems if purified recombinant proteins are to be included in a vaccine. The mode of presentation of a recombinant antigen to the immune system can also be an important factor in the immune response.

Nucleic acid vaccination has been shown to induce protective immune responses in non-viral systems and in diverse animal species (Special Conference Issue, WHO meeting on nucleic acid vaccines [1994] Vaccine 12:1491). Nucleic acid vaccination has induced cytotoxic lymphocyte (CTL), T-helper 1, and antibody responses, and has been shown to be protective against disease (Ulmer, J., J. Donelly, S. Parker et al. [1993] Science 259:1745). For example, direct intramuscular injection of mice with DNA encoding the influenza nucleoprotein caused the production of high titer antibodies, nucleoprotein-specific CTLs, and protection against viral challenge. Immunization of mice with plasmid DNA encoding the Plasmodium yoelii circumsporozoite protein induced high antibody titers against malaria sporozoites and CTLs, and protection against challenge infection (Sedegah, M., R. Hedstrom, P. Hobart, S. Hoffman [1994] Proc. Natl. Acad. Sci. USA 91:9866). Cattle immunized with plasmids encoding bovine herpesvirus 1 (BHV-1) glycoprotein IV developed neutralizing antibody and were partially protected (Cox, G., T. Zamb, L. Babiuk [1993] J. Virol. 67:5664). However, it has been a question in the field of immunization whether the recently discovered technology of nucleic acid vaccines can provide improved protection against an antigenic drift variant. Moreover, it has not heretofore been recognized or suggested that nucleic acid vaccines may be successful to protect against rickettsial disease or that a major surface protein conserved in rickettsia was protective against disease.

BRIEF SUMMARY OF THE INVENTION

Disclosed and claimed here are novel vaccines for conferring immunity to rickettsia infection,

including Cowdria ruminantium causing heartwater. Also disclosed are novel nucleic acid compositions and methods of using those compositions, including to confer immunity in a susceptible host. Also disclosed are novel materials and methods for diagnosing infections by Ehrlichia in humans or animals.

One aspect of the subject invention concerns a nucleic acid, e.g., DNA or mRNA, vaccine containing the major antigenic protein 1 gene (MAP1) or the major antigenic protein 2 gene (MAP2) of rickettsial pathogens. In one embodiment, the nucleic acid vaccines can be driven by the human cytomegalovirus (HCMV) enhancer-promoter. In studies immunizing mice by intramuscular injection of a DNA vaccine composition according to the subject invention, immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with vector only, proliferated in response to recombinant MAP1 and rickettsial antigens in in vitro lymphocyte proliferation tests. In experiments testing different DNA vaccine dose regimens, increased survival rates as compared to controls were observed on challenge with rickettsia. Accordingly, the subject invention concerns the discovery that DNA vaccines can induce protective immunity against rickettsial disease or death resulting therefrom.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C show a comparison of the amino acid sequences from alignment of the three rickettsial proteins, namely, Cowdria ruminantium (C.r.), Ehrlichia chaffeensis (E.c.), and Anaplasma marginale (A.m.).

FIGS. 2A-2C shows the DNA sequence of the 28 kDa gene locus cloned from E. chaffeensis (FIGS. 2A-2B) and E. canis (FIG. 2C). One letter amino acid codes for the deduced protein sequences are presented below the nucleotide sequence. The proposed sigma-70-like promoter sequences (38) are presented in bold and underlined text as -10 and -35 (consensus -35 and -10 sequences are TTGACA and TATAAT, respectively). Similarly, consensus ribosomal binding sites and transcription terminator sequences (bold letter sequence) are identified. G-rich regions identified in the E. chaffeensis sequence are underlined. The conserved sequences from within the coding regions selected for RT-PCR assay are identified with italics and underlined text.

FIG. 3A shows the complete sequence of the MAP2 homolog of Ehrlichia canis. The arrow (.fwdarw.) represents the predicted start of the mature protein. The asterisk (*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

FIG. 3B shows the complete sequence of the MAP2 homolog of Ehrlichia chaffeensis. The arrow (.fwdarw.) represents the predicted start of the mature protein. The asterisk (*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO. 1 is the coding sequence of the MAP1 gene from Cowdria ruminantium (Highway isolate).

SEQ ID NO. 2 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 1.

SEQ ID NO. 3 is the coding sequence of the MAP1 gene from Ehrlichia chaffeensis.

SEQ ID NO. 4 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 3.

SEQ ID NO. 5 is the Anaplasma marginale MSP4 gene coding sequence.

SEQ ID NO. 6 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 5.

SEQ ID NO. 7 is a partial coding sequence of the VSA1 gene from Ehrlichia chaffeensis, also shown in FIGS. 2A-2B.

SEQ ID NO. 8 is the coding sequence of the VSA2 gene from Ehrlichia chaffeensis, also shown in FIGS. 2A-2B.

SEQ ID NO. 9 is the coding sequence of the VSA3 gene from Ehrlichia chaffeensis, also shown in FIGS. 2A-2B.

SEQ ID NO. 10 is the coding sequence of the VSA4 gene from Ehrlichia chaffeensis, also shown in FIGS. 2A-2B.

SEQ ID NO. 11 is a partial coding sequence of the VSA5 gene from Ehrlichia chaffeensis, also shown in FIGS. 2A-2B.

SEQ ID NO. 12 is the coding sequence of the VSA1 gene from Ehrlichia canis, also shown in FIG. 2C.

SEQ ID NO. 13 is a partial coding sequence of the VSA2 gene from Ehrlichia canis, also shown in FIG. 2C.

SEQ ID NO. 14 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 7, also shown in FIGS. 2A-2B.

SEQ ID NO. 15 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 8, also shown in FIGS. 2A-2B.

SEQ ID NO. 16 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 9, also shown in FIGS. 2A-2B.

SEQ ID NO. 17 is the polypeptide encoded by the polynuceotide of SEQ ID NO. 10, also shown in FIGS. 2A-2B.

SEQ ID NO. 18 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 11, also shown

in FIGS. 2A-2B.

SEQ ID NO. 19 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 12, also shown in FIG. 2C.

SEQ ID NO. 20 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 13, also shown in FIG. 2C.

SEQ ID NO. 21 is the coding sequence of the MAP2 gene from Ehrlichia canis, also shown in FIG. 3A.

SEQ ID NO. 22 is the coding sequence of the MAP2 gene from Ehrlichia chaffeensis, also shown in FIG. 3B.

SEQ ID NO. 23 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 21, also shown in FIG. 3A.

SEQ ID NO. 24 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 22, also shown in FIG. 3B.

DETAILED DISCLOSURE OF THE INVENTION

In one embodiment, the subject invention concerns a novel strategy, termed nucleic acid vaccination, for eliciting an immune response protective against rickettsial disease. The subject invention also concerns novel compositions that can be employed according to this novel strategy for eliciting a protective immune response. According to the subject invention, recombinant plasmid DNA or mRNA encoding an antigen of interest is inoculated directly into the human or animal host where the antigen is expressed and an immune response induced. Advantageously, problems of protein purification, as can be encountered with antigen delivery using live vectors, can be virtually eliminated by employing the compositions or methods according to the subject invention. Unlike live vector delivery, the subject invention can provide a further advantage in that the DNA or RNA does not replicate in the host, but remains episomal with gene expression directed for as long as 19 months or more post-injection. See, for example, Wolff, J. A., J. J. Ludike, G. Acsadi, P. Williams, A. Jani (1992) Hum. Mol. Genet. 1:363. A complete immune response can be obtained as recombinant antigen is synthesized intracellularly and presented to the host immune system in the context of autologous class I and class II MHC molecules.

In one embodiment, the subject invention concerns nucleic acids and compositions comprising those nucleic acids that can be effective in protecting an animal from disease or death caused by rickettsia. For example, a nucleic acid vaccine of the subject invention has been shown to be protective against Cowdria ruminantium, the causative agent of heartwater in domestic ruminants. Accordingly, DNA sequences of rickettsial genes, e.g, MAP1 or homologues thereof, can be used as nucleic acid vaccines against human and animal rickettsial diseases. The MAP1 gene used to obtain this protection is also present in other rickettsiae including Anaplasma marginale, Ehrlichia canis, and in a causative agent of human ehrlichiosis, Ehrlichia chaffeensis (van Vliet, A., F. Jongejan, M. van Kleef, B. van der Zeijst [1994] Infect. Immun. 62:1451). The MAP1 gene or a MAP1-like gene can also be found in certain Rickettsia spp. MAP1-like genes

from Ehrlichia chaffeensis and Ehrlichia canis have now been cloned and sequenced. These MAP-1 homologs are also referred to herein as Variable Surface Antigen (VSA) genes.

The present invention also concerns polynucleotides encoding MAP2 or MAP2 homologs from Ehrlichia canis and Ehrlichia chaffeensis. MAP2 polynucleotide sequences of the invention can be used as vaccine compositions and in diagnostic assays. The polynucleotides can also be used to produce the MAP2 polypeptides encoded thereby.

Compositions comprising the subject polynucleotides can include appropriate nucleic acid vaccine vectors (plasmids), which are commercially available (e.g., Vical, San Diego, Calif.). In addition, the compositions can include a pharmaceutically acceptable carrier, e.g., saline. The pharmaceutically acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E. W. Martin's Remington's Pharmaceutical Science, Mack Publishing Company, Easton, Pa.

The subject invention also concerns polypeptides encoded by the subject polynucleotides. Specifically exemplified are the polypeptides encoded by the MAP-1 and VSA genes of C. rumimontium, E. chaffeensis, E. canis and the MP4 gene of Anaplasma marginale. Polypeptides encoded by E. chaffeensis and E. canis MAP2 genes are also exemplified herein.

Also encompassed within the scope of the present invention are fragments and variants of the exemplified polynucleotides. Variants include polynucleotides and/or polypeptides having base or amino acid additions, deletions and substitutions in the sequence of the subject molecule so long as those variants have substantially the same activity or serologic reactivity as the native molecules. Also included are allelic variants of the subject polynucleotides. The polypeptides and peptides of the present invention can be used to raise antibodies that are reactive with the polypeptides disclosed herein. The polypeptides and peptides can also be used as molecular weight markers.

Another aspect oft he subject invention concerns antibodies reactive with MAP-1 and MAP2 polypeptides disclosed herein. Antibodies can be monoclonal or polyclonal and can be produced using standard techniques known in the art. Antibodies of the invention can be used in diagnostic and therapeutic applications.

In a specific embodiment, the subject invention concerns a DNA vaccine (e.g., VCL1010/MAP1) containing the major antigenic protein 1 gene (MAP1) driven by the human cytomegalovirus (HCMV) enhancer-promoter injected intramuscularly into 8-10 week-old female DBA/2 mice after treating them with 50 .mu.l/muscle of 0.5% bupivacaine 3 days previously. Up to 75% of the VCL1010/MAP1-immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with VCLO1010 DNA (plasmid vector, Vical, San Diego) proliferated in response to recombinant MAP1 and C. ruminantium antigens in in vitro lymphocyte proliferation tests. These proliferating cells from mice immunized with VCL1010/MAP1 DNA secreted IFN-gamma and IL-2 at concentrations ranging from 610 pg/ml and 152 pg/ml to 1290 pg/ml and 310 pg/ml, respectively. In experiments testing different VCL1010/MAP1 DNA vaccine dose regimens (25-100 .mu.g/dose, 2 or 4 immunizations), survival rates of 23% to 88% (35/92 survivors/total in all VCL1010/MAP1 immunized groups) were observed on challenge with 30LD50 of C. ruminantium. Survival rates of 0% to 3% (1/144 survivors/total in all control groups) were

recorded for control mice immunized similarly with VCL1010 DNA or saline. Accordingly, the subject invention concerns the discovery that the gene encoding the MAP1 protein can induce protective immunity as a DNA vaccine against rickettsial disease.

The nucleic acid sequences described herein have other uses as well. For example, the nucleic acids of the subject invention can be useful as probes to identify complementary sequences within other nucleic acid molecules or genomes. Such use of probes can be applied to identify or distinguish infectious strains of organisms in diagnostic procedures or in rickettsial research where identification of particular organisms or strains is needed. As is well known in the art, probes can be made by labeling the nucleic acid sequences of interest according to accepted nucleic acid labeling procedures and techniques. A person of ordinary skill in the art would recognize that variations or fragments of the disclosed sequences which can specifically and selectively hybridize to the DNA of rickettsia can also function as a probe. It is within the ordinary skill of persons in the art, and does not require undue experimentation in view of the description provided herein, to determine whether a segment of the claimed DNA sequences is a fragment or variant which has characteristics of the full sequence, e.g., whether it specifically and selectively hybridizes or can confer protection against rickettsial infection in accordance with the subject invention. In addition, with the benefit of the subject disclosure describing the specific sequences, it is within the ordinary skill of those persons in the art to label hybridizing sequences to produce a probe.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, Bal31 exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York; Wei et al. (1983) J. Biol. Chem. 258:13006-13512.

In addition, the nucleic acid sequences of the subject invention can be used as molecular weight markers in nucleic acid analysis procedures.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1

A nucleic acid vaccine construct was tested in animals for its ability to protect against death caused by infection with the rickettsia Cowdria ruminantium. The vaccine construct tested was the MAP1 gene of C. ruminantium inserted into plasmid VCL1010 (Vical, San Diego) under control of the human cytomegalovirus promoter-enhancer and intron A. In this study, seven groups containing 10 mice each were injected twice at 2-week intervals with either 100, 75, 50, or 25 .mu.g VCL1001/MAP1 DNA (V/M in Table 1 below), or 100, 50 .mu.g VCL1010 DNA (V in Table 1) or saline (Sal.), respectively. Two weeks after the last injections, 8 mice/group were challenged with 30LD50 of C. ruminantium and clinical symptoms and survival monitored. The remaining 2 mice/group were not challenged and were used for lymphocyte proliferation tests and cytokine measurements. The results of the study are summarized in Table 1, below:

TABLE 1

	100 .mu.g V/M	75 .mu.g V/M	50 .mu.g V/M	25 .mu.g V/M	100 .mu.g V	50 .mu.g V	.mu.g Sal.
Survived	5	7	5	3	0	0	0
Died	3	1	3	5	8	8	8

The VCL1010/MAP1 nucleic acid vaccine increased survival on challenge in all groups, with a total of 20/30 mice surviving compared to 0/24 in the control groups.

This study was repeated with another 6 groups, each containing 33 mice (a total of 198 mice). Three groups received 75 .mu.g VCL1010/MAP1 DNA or VCL1010 DNA or saline (4 injections in all cases). Two weeks after the last injection,30 mice/group were challenged with 30LD50 of C. ruminantium and 3 mice/group were sacrificed for lymphocyte proliferation tests and cytokine measurements. The results of this study are summarized in Table 2, below:

TABLE 2

	V/M 2 inj.	V 2 inj.	Sal. 2 inj.	V/M 4 inj.	V 4 inj.	Sal. 4 inj.
Survived	7	0	0	8	0	1
Died*	23	30	30	22	30	29

*In mice that died in both V/M groups, there was an increase in mean survival time of approximately 4 days compared to the controls ($p < 0.05$)

Again, as summarized in Table 2, the VCLlO1010/MAP1 DNA vaccine increased the numbers of mice surviving in both immunized groups, although there was no apparent benefit of 2 additional injections. In these two experiments, there were a cumulative total of 35/92 (38%) surviving mice in groups receiving the VCL1010/MAP1 DNA vaccine compared to 1/144 (0.7%) surviving mice in the control groups. In both immunization and challenge trials described above, splenocytes from VCL1010/MAP1 immunized mice, but not from control mice, specifically proliferated to recombinant MAP1 protein and to C. ruminantium in lymphocyte proliferation tests. These proliferating splenocytes secreted IL-2 and gamma-interferon at concentrations up to 310 and 1290 pg/ml respectively. These data show that protection against rickettsial infections can be achieved with a DNA vaccine. In addition, these experiments show MAP1-related proteins as vaccine targets.

Example 2

The MAP1 protein of C. ruminantium has significant similarity to MSP4 of A. marginale, and related molecules may also be presenting other rickettsial pathogens. To prove this, we used primers based on regions conserved between C. ruminantium and A. marginale in PCR to clone a MAP1-like gene from E. chaffeensis. The amino acid sequence derived from the cloned E. chaffeensis MAP1-like gene, and alignment with the corresponding genes of C. ruminantium and A. marginale is shown in FIG. 1. We have now identified the regions of MAP1-like genes which are highly conserved between Ehrlichia, Cowdria, and Anaplasma and which can allow cloning of the analogous genes from other rickettsiae. Example 3

Cloning and sequence analysis of MAP1 homologue genes of E. chaffeensis and E. canis

Genes homologous to the major surface protein of C. ruminantium MAP1 were cloned from E. chaffeensis and E. canis by using PCR cloning strategies. The cloned segments represent a 4.6 kb genomic locus of E. chaffeensis and a 1.6 kb locus of E. canis. DNA sequence generated from these clones was assembled and is presented along with the deduced amino acid sequence in FIGS. 2A-2B (SEQ ID NOS. 7-11 and 14-18) and FIG. 2C (SEQ ID NOS. 12-13 and 19-20). Significant features of the DNA include five very similar but nonidentical open reading frames (ORFs) for E. chaffeensis and two very similar, nonidentical ORFs for the E. canis cloned locus. The ORFs for both Ehrlichia spp. are separated by noncoding sequences ranging from 264 to 310 base pairs. The noncoding sequences have a higher A+T content (71.6% for E. chaffeensis and 76.1% for E. canis) than do the coding sequences (63.5% for E. chaffeensis and 68.0% for E. canis). A G-rich region -200 bases upstream from the initiation codon, sigma-70-like promoter sequences, putative ribosome binding sites (RBS), termination codons, and palindromic sequences near the termination codons are found in each of the E. chaffeensis noncoding sequences. The E. canis noncoding sequence has the same feature except for the G-rich region (FIG. 2C; SEQ ID NOS. 12-13 and 19-20).

Sequence comparisons of the ORFs at the nucleotide and translated amino acid levels revealed a high degree of similarity between them. The similarity spanned the entire coding sequences, except in three regions where notable sequence variations were observed including some deletions/insertions (Variable Regions I, II and III). Despite the similarities, no two ORFs are identical. The cloned ORF 2, 3 and 4 of E. chaffeensis have complete coding sequences. The ORF1 is a partial gene having only 143 amino acids at the C-terminus whereas the ORF5 is nearly complete but lacks 5-7 amino acids and a termination codon. The cloned ORF2 of E. canis also is a partial gene lacking a part of the C-terminal sequence. The overall similarity between different ORFs at the amino acid level is 56.0% to 85.4% for E. chaffeensis, whereas for E. canis it is 53.3%. The similarity of E. chaffeensis ORFs to the MAP1 coding sequences reported for C. ruminantium isolates ranged from 55.5% to 66.7%, while for E. canis to C. ruminantium it is 48.5% to 54.2%. Due to their high degree of similarity to MAP1 surface antigen genes of C. ruminantium and since they are nonidentical to each other, the E. chaffeensis and E. canis ORFs are referred to herein as putative Variable Surface Antigen (VSA) genes. The apparent molecular masses of the predicted mature proteins of E. chaffeensis were 28.75 kDa for VSA2, 27.78 for VSA3, and 27.95 for VSA4, while E. canis VSA1 was slightly higher at 29.03 kDa. The first 25 amino acids in each VSA coding sequence were eliminated when calculating the protein size since they markedly resembled the signal sequence of C. ruminantium MAP1 and presumably would be absent from the mature protein. Predicted protein sizes for E. chaffeensis VSA1 and VSA5, and E. canis VSA2 were not calculated since the complete genes were not cloned.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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gga aaa gga aac tac cct gca ata gta ata ctg gat gta tgc cac ttt      816
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Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Asp Ala Lys Ala Ser
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His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly
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acgagagcat acctttgtct ccttacatat gtgcaggtgt tggtactgat ttaatatcca 600
tgtttgaagc tacaaatcct aaaatttctt accaagggaa gttaggtcta agttactcta 660
taaacccaga agcttctgta tttattggtg gacattttca taaggtgata ggaaacgaat 720
ttagggacat tcctactctg aaagcatttg ttacgtcatc agctactcca gatctagcaa 780
tagtaacact aagtgtatgt cattttggaa tagaacttgg aggaaggttt aacttct 837
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 10
<211> LENGTH: 843
<212> TYPE: DNA
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE: 10
atatgaattg caaaaaattt tttataacaa ctacattagt atcgctaatg tccttcttac 60
ctggaatatc attttctgat gcagtacaga acgacaatgt tggtggtaat ttctatatca 120
gtgggaaata tgtaccaagt gtttcacatt ttggcgtatt ctctgctaaa caggaaagaa 180
atacaacaat cggagtattt ggattaaagc aagattggga tggcagcaca atatctaaaa 240
attctccaga aaatacattt aacgttccaa attattcatt taaatatgaa aataatccat 300
ttctaggttt tgcaggagct gttggttatt taatgaatgg tccaagaata gagttagaaa 360
tgtcctatga aacatttgat gtgaaaaacc agggtaataa ctataagaac gatgctcaca 420
aatattatgc tttaacccat aacagtgggg gaaagctaag caatgcaggt gataagtttg 480
tttttctaaa aaatgaagga ctacttgata tatcacttat gttgaatgca tgctatgatg 540
taataagtga aggaatacct ttctctcctt acatatgtgc aggtgttggt actgatttaa 600
tatccatgtt tgaagctata aaccctaaaa tttcttatca aggaaagtta ggtttgagtt 660
actccataag cccagaagct tctgtttttg ttggtggaca ttttcataag gtgataggga 720
atgaattcag agatattcct gctatgatac ccagtacctc aactctcaca ggtaatcact 780
ttactatagt aacactaagt gtatgccact ttggagtgga acttggagga aggtttaact 840
ttt 843
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 11
<211> LENGTH: 830
<212> TYPE: DNA
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE: 11
atatgaatta caaaaaagtt ttcataacaa gtgcattgat atcattaata tcttctctac 60
ctggagtatc attttccgac ccagcaggta gtggtattaa cggtaatttc tacatcagtg 120
gaaaatacat gccaagtgct tcgcattttg gagtattctc tgctaaggaa gaaagaaata 180
caacagttgg agtgtttgga ctgaagcaaa attgggacgg aagcgcaata tccaactcct 240
ccccaaacga tgtattcact gtctcaaatt attcatttaa atatgaaaac aacccgtttt 300
taggttttgc aggagctatt ggttactcaa tggatggtcc aagaatagag cttgaagtat 360
cttatgaaac atttgatgta aaaaatcaag gtaacaatta taagaatgaa gcacatagat 420
attgtgctct atcccataac tcagcagcag acatgagtag tgcaagtaat aattttgtct 480
ttctaaaaaa tgaaggatta cttgacatat catttatgct gaacgcatgc tatgacgtag 540

taggcgaagg catacctttt tctccttata tatgcgcagg tatcggtact gatttagtat 600
ccatgtttga agctacaaat cctaaaattt cttaccaagg aaagttaggt ttaagctact 660
ctataagccc agaagcttct gtgtttattg gtgggcactt tcataaggta atagggaacg 720
aatttagaga tattcctact ataataccta ctggatcaac acttgcagga aaaggaaact 780
accctgcaat agtaatactg gatgtatgcc actttggaat agaaatggga 830

<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 12
<211> LENGTH: 864
<212> TYPE: DNA
<213> ORGANISM: Ehrlichia canis
<400> SEQUENCE: 12

atatgaaata taaaaaaact tttacagtaa ctgcattagt attattaact tcctttacac 60
attttatacc tttttatagt ccagcacgtg ccagtacaat tcacaacttc tacattagtg 120
gaaaatatat gccaacagcg tcacattttg gaatttttc agctaaagaa gaacaaagtt 180
ttactaaggt attagttggg ttagatcaac gattatcaca taatattata aacaataatg 240
atacagcaaa gagtcttaag gttcaaaatt attcatttaa atacaaaaat aacccatttc 300
taggatttgc aggagctatt ggttattcaa taggcaattc aagaatagaa ctagaagtat 360
cacatgaaat atttgatact aaaaacccag gaaacaatta tttaaatgac tctcacaaat 420
attgcgcttt atctcatgga agtcacatat gcagtgatgg aaatagcgga gattggtaca 480
ctgcaaaaac tgataagttt gtacttctga aaaatgaagg tttacttgac gtctcattta 540
tgttaaacgc atgttatgac ataacaactg aaaaaatgcc tttttcacct tatatatgtg 600
caggtattgg tactgatctc atatctatgt ttgagacaac acaaaacaaa atatcttatc 660
aaggaaagtt aggtttaaac tatactataa actcaagagt ttctgttttt gcaggtgggc 720
actttcataa ggtaataggt aatgaattta aaggtattcc tactctatta cctgatggat 780
caaacattaa agtacaacag tctgcaacag taacattaga tgtgtgccat ttcgggttag 840
agattggaag tagatttttc tttt 864

<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 13
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Ehrlichia canis
<400> SEQUENCE: 13

atatgaattg taaaaaagtt ttcacaataa gtgcattgat atcatccata tacttcctac 60
ctaatgtctc atactctaac ccagtatatg gtaacagtat gtatggtaat ttttacatat 120
caggaaagta catgccaagt gttcctcatt ttggaatttt ttcagctgaa gaagagaaaa 180
aaaagacaac tgtagtatat ggcttaaaag aaaactgggc aggagatgca atatctagtc 240
aaagtccaga tgataatttt accattcgaa attactcatt caagtatgca agcaacaagt 300
ttttagggtt tgcagtagct attggttact cgataggcag tccaagaata gaagttgaga 360
tgtcttatga agcatttgat gtaaaaaatc aaggtaaca 399

<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 14
<211> LENGTH: 43
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE: 14

Asn Glu Phe Arg Asp Ile Ser Thr Leu Lys Ala Phe Ala Thr Pro Ser
1 5 10 15
Ser Ala Ala Thr Pro Asp Leu Ala Thr Val Thr Leu Ser Val Cys His
20 25 30
Phe Gly Val Glu Leu Gly Gly Arg Phe Asn Phe
35 40

<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 15
<211> LENGTH: 286
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE: 15

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Met Asn Cys Glu Lys Phe Phe Ile Thr Thr Ala Leu Thr Leu Leu Met
  1               5                  10                  15
Ser Phe Leu Pro Gly Ile Ser Leu Ser Asp Pro Val Gln Asp Asp Asn
             20                  25                  30
Ile Ser Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser
         35                  40                  45
His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly
     50                  55                  60
Val Phe Gly Ile Glu Gln Asp Trp Asp Arg Cys Val Ile Ser Arg Thr
 65                  70                  75                  80
Thr Leu Ser Asp Ile Phe Thr Val Pro Asn Tyr Ser Phe Lys Tyr Glu
                 85                  90                  95
Asn Asn Leu Phe Ser Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp
            100                 105                 110
Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Ala Phe Asp Val Lys
        115                 120                 125
Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Tyr Ala Leu
    130                 135                 140
Ser His Leu Leu Gly Thr Glu Thr Gln Ile Asp Gly Ala Gly Ser Ala
145                 150                 155                 160
Ser Val Phe Leu Ile Asn Glu Gly Leu Leu Asp Lys Ser Phe Met Leu
                165                 170                 175
Asn Ala Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr
            180                 185                 190
Ile Cys Ala Gly Ile Gly Ile Asp Leu Val Ser Met Phe Glu Ala Ile
        195                 200                 205
Asn Pro Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Pro Ile
    210                 215                 220
Ser Pro Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile
225                 230                 235                 240
Gly Asn Glu Phe Arg Asp Ile Pro Thr Met Ile Pro Ser Glu Ser Ala
                245                 250                 255
Leu Ala Gly Lys Gly Asn Tyr Pro Ala Ile Val Thr Leu Asp Val Phe
            260                 265                 270
Tyr Phe Gly Ile Glu Leu Gly Gly Arg Phe Asn Phe Gln Leu
        275                 280                 285
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 16
<211> LENGTH: 278
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE:  16
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Val Ser Leu Met
  1               5                  10                  15
Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Pro Val Gln Gly Asp Asn
             20                  25                  30
Ile Ser Gly Asn Phe Tyr Val Ser Gly Lys Tyr Met Pro Ser Ala Ser
         35                  40                  45

His Phe Gly Met Phe Ser Ala Lys Glu Glu Lys Asn Pro Thr Val Ala
     50                  55                  60
Leu Tyr Gly Leu Lys Gln Asp Trp Glu Gly Ile Ser Ser Ser Ser His
 65                  70                  75                  80
Asn Asp Asn His Phe Asn Asn Lys Gly Tyr Ser Phe Lys Tyr Glu Asn
                 85                  90                  95
Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Gly Gly
            100                 105                 110
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Pro Arg Val Glu Phe Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn
        115                 120                 125
Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Arg Tyr Cys Ala Leu Gly
    130                 135                 140
Gln Gln Asp Asn Ser Gly Ile Pro Lys Thr Ser Lys Tyr Val Leu Leu
145                 150                 155                 160
Lys Ser Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys Tyr
                165                 170                 175
Asp Ile Ile Asn Glu Ser Ile Pro Leu Ser Pro Tyr Ile Cys Ala Gly
            180                 185                 190
Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Thr Asn Pro Lys Ile
        195                 200                 205
Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Asn Pro Glu Ala
    210                 215                 220
Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu Phe
225                 230                 235                 240
Arg Asp Ile Pro Thr Leu Lys Ala Phe Val Thr Ser Ser Ala Thr Pro
                245                 250                 255
Asp Leu Ala Ile Val Thr Leu Ser Val Cys His Phe Gly Ile Glu Leu
            260                 265                 270
Gly Gly Arg Phe Asn Phe
        275
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 17
<211> LENGTH: 280
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE:  17
Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Thr Leu Val Ser Leu Met
  1               5                  10                  15
Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Ala Val Gln Asn Asp Asn
             20                  25                  30
Val Gly Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Val Pro Ser Val Ser
         35                  40                  45
His Phe Gly Val Phe Ser Ala Lys Gln Glu Arg Asn Thr Thr Ile Gly
     50                  55                  60
Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ser Thr Ile Ser Lys Asn
 65                  70                  75                  80
Ser Pro Glu Asn Thr Phe Asn Val Pro Asn Tyr Ser Phe Lys Tyr Glu
                 85                  90                  95
Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Val Gly Tyr Leu Met Asn
            100                 105                 110
Gly Pro Arg Ile Glu Leu Glu Met Ser Tyr Glu Thr Phe Asp Val Lys
        115                 120                 125
Asn Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Lys Tyr Tyr Ala Leu
    130                 135                 140
Thr His Asn Ser Gly Gly Lys Leu Ser Asn Ala Gly Asp Lys Phe Val
145                 150                 155                 160
Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Leu Met Leu Asn Ala
                165                 170                 175
Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys
            180                 185                 190
Ala Gly Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Ile Asn Pro
        195                 200                 205
Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro
    210                 215                 220
Glu Ala Ser Val Phe Val Gly Gly His Phe His Lys Val Ile Gly Asn
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225                 230                 235                 240
Glu Phe Arg Asp Ile Pro Ala Met Ile Pro Ser Thr Ser Thr Leu Thr
                245                 250                 255
Gly Asn His Phe Thr Ile Val Thr Leu Ser Val Cys His Phe Gly Val
            260                 265                 270
Glu Leu Gly Gly Arg Phe Asn Phe
        275                 280
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 18
<211> LENGTH: 276
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE:  18
Met Asn Tyr Lys Lys Val Phe Ile Thr Ser Ala Leu Ile Ser Leu Ile
  1               5                  10                  15
Ser Ser Leu Pro Gly Val Ser Phe Ser Asp Pro Ala Gly Ser Gly Ile
             20                  25                  30
Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser His
         35                  40                  45
Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly Val
     50                  55                  60
Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser Ser
 65                  70                  75                  80
Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu Asn
                85                  90                  95
Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp Gly
            100                 105                 110
Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn
        115                 120                 125
Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu Ser
    130                 135                 140
His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val Phe
145                 150                 155                 160
Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys
                165                 170                 175
Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys Ala
            180                 185                 190
Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro Lys
        195                 200                 205
Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro Glu
    210                 215                 220
Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu
225                 230                 235                 240
Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala Gly
                245                 250                 255
Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe Gly
            260                 265                 270
Ile Glu Met Gly
        275
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 19
<211> LENGTH: 287
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia canis
<400> SEQUENCE:  19
Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu Val Leu Leu Thr
  1               5                  10                  15
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Ser Phe Thr His Phe Ile Pro Phe Tyr Ser Pro Ala Arg Ala Ser Thr
             20                  25                  30
Ile His Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Thr Ala Ser His
         35                  40                  45
Phe Gly Ile Phe Ser Ala Lys Glu Glu Gln Ser Phe Thr Lys Val Leu
     50                  55                  60
Val Gly Leu Asp Gln Arg Leu Ser His Asn Ile Ile Asn Asn Asn Asp
 65                  70                  75                  80
Thr Ala Lys Ser Leu Lys Val Gln Asn Tyr Ser Phe Lys Tyr Lys Asn
                 85                  90                  95
Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Ile Gly Asn
            100                 105                 110
Ser Arg Ile Glu Leu Glu Val Ser His Glu Ile Phe Asp Thr Lys Asn
        115                 120                 125
Pro Gly Asn Asn Tyr Leu Asn Asp Ser His Lys Tyr Cys Ala Leu Ser
    130                 135                 140
His Gly Ser His Ile Cys Ser Asp Gly Asn Ser Gly Asp Trp Tyr Thr
145                 150                 155                 160
Ala Lys Thr Asp Lys Phe Val Leu Leu Lys Asn Glu Gly Leu Leu Asp
                165                 170                 175
Val Ser Phe Met Leu Asn Ala Cys Tyr Asp Ile Thr Thr Glu Lys Met
            180                 185                 190
Pro Phe Ser Pro Tyr Ile Cys Ala Gly Ile Gly Thr Asp Leu Ile Ser
        195                 200                 205
Met Phe Glu Thr Thr Gln Asn Lys Ile Ser Tyr Gln Gly Lys Leu Gly
    210                 215                 220
Leu Asn Tyr Thr Ile Asn Ser Arg Val Ser Val Phe Ala Gly Gly His
225                 230                 235                 240
Phe His Lys Val Ile Gly Asn Glu Phe Lys Gly Ile Pro Thr Leu Leu
                245                 250                 255
Pro Asp Gly Ser Asn Ile Lys Val Gln Gln Ser Ala Thr Val Thr Leu
            260                 265                 270
Asp Val Cys His Phe Gly Leu Glu Ile Gly Ser Arg Phe Phe Phe
        275                 280                 285
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 20
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia canis
<400> SEQUENCE:  20
Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala Leu Ile Ser Ser Ile
  1               5                  10                  15
Tyr Phe Leu Pro Asn Val Ser Tyr Ser Asn Pro Val Tyr Gly Asn Ser
             20                  25                  30
Met Tyr Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Val Pro
         35                  40                  45
His Phe Gly Ile Phe Ser Ala Glu Glu Glu Lys Lys Lys Thr Thr Val
     50                  55                  60
Val Tyr Gly Leu Lys Glu Asn Trp Ala Gly Asp Ala Ile Ser Ser Gln
 65                  70                  75                  80
Ser Pro Asp Asp Asn Phe Thr Ile Arg Asn Tyr Ser Phe Lys Tyr Ala
                 85                  90                  95
Ser Asn Lys Phe Leu Gly Phe Ala Val Ala Ile Gly Tyr Ser Ile Gly
            100                 105                 110
Ser Pro Arg Ile Glu Val Glu Met Ser Tyr Glu Ala Phe Asp Val Lys
        115                 120                 125
Asn Gln Gly Asn Asn
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130
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 21
<211> LENGTH: 686
<212> TYPE: DNA
<213> ORGANISM: Ehrlichia canis
<400> SEQUENCE: 21
atgaaagcta tcaaattcat acttaatgtc tgcttactat ttgcagcaat atttttaggg 60
tattcctata ttacaaaaca aggcatattt caaacaaaac atcatgatac acctaatact 120
actataccaa atgaagacgg tattcaatct agctttagct taatcaatca agacggtaaa 180
acagtaacca gccaagattt cctagggaaa cacatgttag ttttgtttgg attctctgca 240
tgtaaaagca tttgccctgc agaattggga ttagtatctg aagcacttgc acaacttggt 300
aataatgcag acaaattaca agtaattttt attacaattg atccaaaaaa tgatactgta 360
gaaaaattaa aagaatttca tgaacatttt gattcaagaa ttcaaatgtt aacaggaaat 420
actgaagaca ttaatcaaat aattaaaaat tataaaatat atgttggaca agcagataaa 480
gatcatcaaa ttaaccattc tgcaataatg taccttattg acaaaaaagg atcatatctt 540
tcacacttca ttccagattt aaaatcacaa gaaaatcaag tagataagtt actatcttta 600
gttaagcagt atctgtaaat aaattcatgg aatacgttgg atgagtaggt ttttttagt 660
atttttagtg ctaataacat tggcat 686
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 22
<211> LENGTH: 618
<212> TYPE: DNA
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE: 22
atgaaagtta tcaaatttat acttaatatc tgtttattat ttgcagcaat ttttctagga 60
tattcctacg taacaaaaca aggcattttt caagtaagag atcataacac tcccaataca 120
aatatatcaa ataaagccag cattactact agtttttcgt tagtaaatca agatggaaat 180
acagtaaata gtcaagattt tttgggaaaa tacatgctag ttttatttgg attttcttca 240
tgtaaaagca tctgccctgc tgaattagga atagcatctg aagttctctc acagcttggt 300
aatgacacag acaagttaca agtaattttc attacaattg atccaacaaa tgatactgta 360
caaaaattaa aaacatttca tgaacatttt gatcctagaa ttcaaatgct aacaggcagt 420
gcagaagata ttgaaaaaat aataaaaaat tacaaaatat atgttggaca agcagataaa 480
gataatcaaa ttgatcactc tgccataatg tacattatcg ataaaaaagg agaatacatt 540
tcacactttt ctccagattt aaaatcaaca gaaaatcaag tagataagtt actatctata 600
ataaaacaat atctctaa 618
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 23
<211> LENGTH: 205
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia canis
<400> SEQUENCE: 23
Met Lys Ala Ile Lys Phe Ile Leu Asn Val Cys Leu Leu Phe Ala Ala
1 5 10 15
Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Thr
20 25 30
Lys His His Asp Thr Pro Asn Thr Thr Ile Pro Asn Glu Asp Gly Ile
35 40 45
Gln Ser Ser Phe Ser Leu Ile Asn Gln Asp Gly Lys Thr Val Thr Ser
50 55 60
Gln Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ala
65 70 75 80
Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Leu Val Ser Glu Ala Leu
85 90 95
Ala Gln Leu Gly Asn Asn Ala Asp Lys Leu Gln Val Ile Phe Ile Thr
100 105 110
Ile Asp Pro Lys Asn Asp Thr Val Glu Lys Leu Lys Glu Phe His Glu

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        115                 120                 125
His Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Thr Glu Asp Ile
    130                 135                 140
Asn Gln Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys
145                 150                 155                 160
Asp His Gln Ile Asn His Ser Ala Ile Met Tyr Leu Ile Asp Lys Lys
                165                 170                 175
Gly Ser Tyr Leu Ser His Phe Ile Pro Asp Leu Lys Ser Gln Glu Asn
            180                 185                 190
Gln Val Asp Lys Leu Leu Ser Leu Val Lys Gln Tyr Leu
        195                 200                 205
<200> SEQUENCE CHARACTERISTICS:
<210> SEQ ID NO 24
<211> LENGTH: 205
<212> TYPE: PRT
<213> ORGANISM: Ehrlichia chaffeensis
<400> SEQUENCE:  24
Met Lys Val Ile Lys Phe Ile Leu Asn Ile Cys Leu Leu Phe Ala Ala
  1               5                  10                  15

Ile Phe Leu Gly Tyr Ser Tyr Val Thr Lys Gln Gly Ile Phe Gln Val
             20                  25                  30
Arg Asp His Asn Thr Pro Asn Thr Asn Ile Ser Asn Lys Ala Ser Ile
         35                  40                  45
Thr Thr Ser Phe Ser Leu Val Asn Gln Asp Gly Asn Thr Val Asn Ser
     50                  55                  60
Gln Asp Phe Leu Gly Lys Tyr Met Leu Val Leu Phe Gly Phe Ser Ser
 65                  70                  75                  80
Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Ile Ala Ser Glu Val Leu
                 85                  90                  95
Ser Gln Leu Gly Asn Asp Thr Asp Lys Leu Gln Val Ile Phe Ile Thr
            100                 105                 110
Ile Asp Pro Thr Asn Asp Thr Val Gln Lys Leu Lys Thr Phe His Glu
        115                 120                 125
His Phe Asp Pro Arg Ile Gln Met Leu Thr Gly Ser Ala Glu Asp Ile
    130                 135                 140
Glu Lys Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys
145                 150                 155                 160
Asp Asn Gln Ile Asp His Ser Ala Ile Met Tyr Ile Ile Asp Lys Lys
                165                 170                 175
Gly Glu Tyr Ile Ser His Phe Ser Pro Asp Leu Lys Ser Thr Glu Asn
            180                 185                 190
Gln Val Asp Lys Leu Leu Ser Ile Ile Lys Gln Tyr Leu
        195                 200                 205
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